

Table 1. Tails of adult geckos at first capture (at Pilliga, N.S.W.). Percentages appear in parentheses.

Period	Adults captured (No.)	Original tails (No.)
<i>Gehyra variegata</i>		
1963-64	282	92 (32.6)
1964-65	101	26 (25.8)
<i>Heteronota binoei</i>		
1963-64	131	55 (42.0)
1964-65	54	15 (27.8)

Gehyra, males 28 percent, females 32 percent; *Heteronota*, males 38 percent, females 43 percent. The slightly greater loss of tails by males of both species probably reflects territorial interactions (3).

Five hundred and ninety six *Gehyra* adults were recaptured as late as 23 months after first capture; 241 *Heteronota* adults, as late as 21 months. By referring each first capture to zero time we could record the proportion of individuals in subsequent monthly assemblages of recaptures that had the same tails as when they were first captured (Table 2).

Graphs of this proportion (on a log scale), against time from first capture, showed the reality of an average rate of autotomy (Figs. 1 and 2). Since the numbers of recaptures after long pe-

Table 2. Numbers of adults that on subsequent recapture had the same tail (original or other) as at first capture.

After first capture (mo)	<i>G. variegata</i> (No.)		<i>H. binoei</i> (No.)	
	Recaptured	Same tails	Recaptured	Same tails
1	72	71	48	48
2	57	56	20	20
3	48	44	13	13
4	40	39	19	15
5	34	32	17	13
6	26	21	20	18
7	33	28	22	18
8	33	27	21	17
9	27	24	10	8
10	29	25	10	8
11	36	26	12	9
12	28	25	10	6
13	17	14	10	5
14	17	12	9	4
15	21	14	14*	5*
16	22	16		
17	12	9		
18	12	9		
19	8	6		
20	8	5		
21	8	4		
22	5	2		
23	3	2		

* 15-20 months combined

riods were fewer than those after short periods, it was necessary to weight the proportions of unchanged tails observed in each monthly cohort according to the total number of animals recaptured.

Because adult individuals were first captured at all seasons of the year, the seasonal variation in rate of tail loss was randomized with respect to time; juveniles were excepted, being more liable to first capture during the summer. At this time the probabilities of tail loss and of predation were at their highest, and about 6 months later they were lowest; for this reason the juvenile data (open circles) were not randomized with respect to season and could not be used in the calculations. Weighted regression coefficients, calculated for the adult data only, were used to estimate an approximate value for the yearly survival of tails. (A more precise solution seems possible.)

Assuming exponential rates of tail loss, we calculated as follows (approximate fiducial limits at the 5-percent level are given in parentheses):

Gehyra variegata: From Fig. 1, the estimated probability of tail survival per year: $p = .774$ (.933, .642). From Table 1, the probability of a gecko having an original tail in 1963-64 (for adult lizards of average age T years): $P = .326$. Assuming a constant rate of tail survival (p) per year, we can derive the average age of adults from $(p)^T = P$; therefore $T = 4.38$ years (15.75, 2.53). Survival in 1964-65 of original tails in adult lizards of average age $(T + 1)$ years: $P = .258$. Similarly then the average age $T + 1 = 5.30$ years (19.10, 3.06).

Heteronota binoei: From Fig. 2, the estimated probability of tail survival per year: $p_1 = .631$ (.772, .516). The probability of original-tail survival recorded during 1963-64 for adult lizards of average age T years was $P_1 = .420$. Again assuming a constant rate of tail survival (p_1) per year, we found the average age of adults to be $T = 1.88$ years (3.32, 1.31). From Table 1, the probability of original-tail survival in 1964-65 for adult lizards of average age $(T + 1)$ years: $P_1 = .278$. Similarly then the average age $T + 1 = 2.79$ years (4.91, 1.94).

For these calculations of the average age of adults we assumed that the rate of tail loss is constant for juvenile and adult individuals (see Figs. 1 and 2). The relative "ages" of the two populations are consistent with the evidence

on longevity available from other sources (4), and the difference between the calculated mean ages of the same individuals in successive years closely approximates 1 year.

Such use of tail-loss data for other species of lizards may be worth consideration, and extensions of the method to other groups of animals, using other types of events, may well be possible.

H. ROBERT BUSTARD

R. D. HUGHES

Department of Zoology, Australian National University, Canberra

References

1. H. G. Cogger, *Proc. Linnean Soc. N.S.W.* **82**, 167 (1957).
2. A. d'A. Bellairs, *Reptiles* (Hutchinson, London, 1957).
3. V. A. Harris, *The Life of the Rainbow Lizard* (Hutchinson, London, 1964).
4. H. R. Bustard, "Ecological studies on Australian lizards," thesis, Australian National Univ., Canberra, 1965.

4 May 1966

Thyroglobulin: Evidence for Crystallization and Association

Abstract. Crystals of thyroglobulin have been obtained from ammonium sulfate solutions and have been examined by electron microscopy as shadowed carbon replicas. Unit size in the crystal is 228 ± 9 angstroms, which corresponds to a molecular weight of 5,300,000. Data are in accord with the possibility that this unit represents a polymer of thyroglobulin.

Crystalline preparations have been obtained of thyroglobulin, the major protein found in aqueous extracts of thyroid tissue. The protein has been studied extensively and is known to have a molecular weight of 660,000 and a frictional ratio of 1.5 (1). It is easily dis-

Table 1. Sedimentation pattern of thyroglobulin at high concentrations of salt. Centrifugation of 2.5 percent thyroglobulin was at 20°C in 1.5M (NH₄)₂SO₄ and 0.015M histidine at pH 7.0. Sedimentation rates are uncorrected; the component sedimenting at 8.1S actually has an $s_{20,w}$ of 19.

Sedimentation rate (S)	Fraction in peak
8.1	0.36
10.0	.28
12.6	.25
Faster*	.11

* The boundary of this component is too broad to permit calculation of a rate but is faster than that of the other components and separable from them.

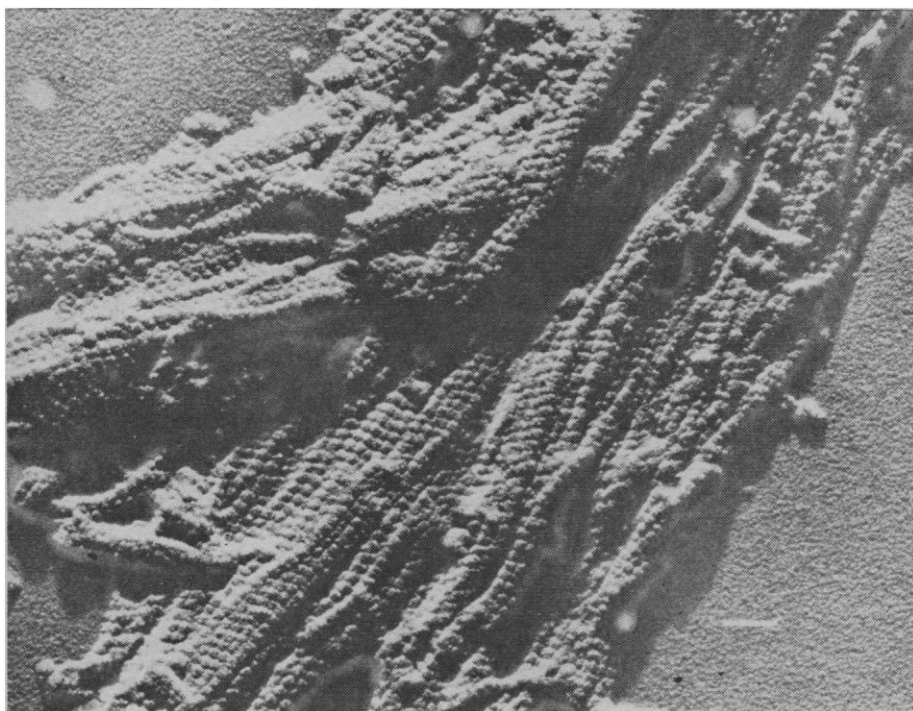


Fig. 1. Paracrystals of thyroglobulin. The white marker is 1000 Å in length. These crystals in saturated $(\text{NH}_4)_2\text{SO}_4$ were deposited on specimen grids and shadowed with platinum.

sociated into subunits with molecular weight of 330,000, and chains of 165,000 are obtainable by quantitative reduction of disulfides in urea or guanidine (2). Another thyroidal iodoprotein, which has a sedimentation rate of 27S and approximately twice the molecular weight of thyroglobulin, appears to have the same basic polypeptide chain structure as this protein (3).

Purified preparations of thyroglobulin were precipitated with ammonium sulfate and sequentially extracted (4) at 0°C with ammonium sulfate solutions of 42, 39, and 37 percent of saturation. After centrifugation the supernatant fluids were set aside at room temperature. Within 30 minutes large quantities, 50 percent or greater in yield, of small crystals were observed with a dark-field microscope in the 39 percent fraction. The method is useful at concentrations of thyroglobulin ranging between 10 and 300 mg/ml. Suspensions were examined in an RCA electron microscope with the technique of shadowed carbon replicas after drying the material on a specimen grid (5). Such preparations (Fig. 1) disclose approximately spherical units, 228 ± 9 Å in diameter, arranged in chains. In many areas the chains are associated side by side. Because of residual salt the fine structure cannot be seen.

The calculation may be made that a protein particle 228 Å in diameter will

have a molecular weight of 5,300,000, assuming a partial specific volume of 0.713. Obviously, a discrepancy exists between the molecular weight as derived in this manner and that calculated from either hydrodynamic or light-scattering experiments. The material with a molecular weight of 660,000, considered as a sphere, should have a diameter of 114 Å. The possibility that particles seen in electron micrographs are aggregates of thyroglobulin was evaluated by examining solutions of the protein in 0.1 percent NaCl by ultracentrifugation under several conditions. Concentrated solutions of protein, as high as 170 mg/ml in 0.1 percent NaCl at pH 6.5, showed no evidence of association. However, in 1.5M ammonium sulfate, three faster components in addition to thyroglobulin were evident (Table 1) which, although not completely resolved, increased in quantity at higher concentrations of protein; the number of new species remained constant with increasing protein concentration. These results suggest that several polymeric forms of thyroglobulin exist in the region in which there is limited solubility of the protein in ammonium sulfate. The unit structure at high concentrations of salt, that is, where crystallization occurs, may represent an octomer of thyroglobulin.

The large size of the particles may represent swelling of thyroglobulin by

salt; it was thought desirable to eliminate this possibility. Hence, a concentrated suspension of crystals was centrifuged at high speed (SW 39 rotor in the Spinco model L centrifuge at 16°C and 40,000 rev/min for 16 hours), and volume of the sediment was measured. When the sediment was dissolved in water, concentration of protein was found to be 525 mg per milliliter of sediment. This value must be corrected for the density of thyroglobulin (1.40) and the packing fraction for closely packed spheres (0.75). Thus, the volume occupied by protein is calculated to be 0.50 ml/ml.

Additional and unknown factors to be considered are the packing efficiency of the crystals themselves and the water of hydration of thyroglobulin, both of which would tend to raise the value for the volume of sediment occupied by protein. Therefore, it seems reasonable to suggest that the spherical particles seen by electron microscopy in crystals of thyroglobulin do represent a polymeric form of the native molecule.

The frictional ratio obtained from hydrodynamic data yields an axial ratio of 9 if the protein is assumed to be a rigid ellipsoid of revolution (1). This value seems too high to be consistent with an approximately spherical structure, whether or not aggregation occurs. But recent results obtained with the use of the technique of polarization of fluorescence yield a relaxation time of 12×10^{-8} second for the protein having a molecular weight of 600,000 (6). This is considerably smaller than a value of 58×10^{-8} second which can be calculated for a rigid ellipsoid of revolution with an axial ratio of 9 and suggests that thyroglobulin has a flexible structure that could more easily assume a spherical shape upon association.

W. B. JAKOBY, L. LABAW

H. EDELHOCH, I. PASTAN, J. E. RALL
*National Institute of Arthritis and
Metabolic Diseases, National Institutes
of Health, Bethesda, Maryland*

References

1. M. Heidelberger and K. O. Pedersen, *J. Gen. Physiol.* **19**, 95 (1935); H. Edelhoch, *J. Biol. Chem.* **235**, 1326 (1960).
2. H. Metzger and H. Edelhoch, *J. Amer. Chem. Soc.* **83**, 1423 (1961); H. Edelhoch and H. Metzger, *ibid.*, p. 1428; B. de Crombrughe, R. Pitt-Rivers, H. Edelhoch, *J. Biol. Chem.* **241**, 2766 (1966).
3. G. Salvatore, G. Vecchio, M. Salvatore, H. J. Cahnmann, J. Robbins, *J. Biol. Chem.* **240**, 2935 (1965).
4. W. B. Jakoby, *Neurospora Newsletter* **8**, 17 (1965).
5. L. Labaw, *J. Ultrastruct. Res.* **10**, 66 (1964).
6. H. Edelhoch and R. F. Steiner, *Biopolymers*, in press.

8 June 1966